REMARKS

Claims 1, 4-8, and 11-16 are pending in the present application.

At the outset, Applicants wish to thank Examiner Baum for the helpful and courteous discussion with their undersigned Representative on October 11, 2005. During this discussion the amendments and remarks set forth herein were discussed. Reconsideration of the remaining rejections is respectfully requested in view of the amendments and remarks set forth herein.

The rejections of Claims 1-11 under 35 U.S.C. §112, first paragraph (written description), are obviated in part by amendment and traversed in part.

The Office has alleged that the specification fails to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. It appears that this ground of rejection is based on the absence of a "representative number of polynucleotide sequence encoding a BZH protein falling within the scope of the claimed genus of polynucleotides which encode the peptide sequence of SEQ ID NO: 5." (page 6, second paragraph of the Office Action mailed July 29, 2005). The Examiner further asserts that Applicants fail to describe structural features common to members of the claimed genus of polynucleotides.

The Examiner is reminded that:

> An objective standard for determining compliance with the written description requirement is, "does the description clearly allow persons of ordinary skill in the art to recognize that he or she invented what is claimed." *In re Gostelli*, 872 F.2d 1008, 1012, 10 USPQ2d 1614, 1618 (Fed. Cir. 1989). (MPEP § 2163.02)

Concomitant with this standard, the Examiner is reminded that:

> Information which is well known in the art need not be described in detail in the specification. See, e.g., *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1379-80, 231 USPQ 81, 90 (Fed. Cir. 1986). (MPEP § 2163)

Applicants submit that the "GRAS family" (also known as the VHIID family) was already known at the time of the present invention. Further, this family is characterized by several conserved motifs. To demonstrate the state of the art that existed at the time of the present invention Applicants **submit herewith** two references discussed on pages 2-3 of the present application as they relate to the description of the GRAS family. These references are:

1) Pysh et al, *The Plant Journal* (1999) **18**(1), 111-119; and

2) Schumacher et al, *Proc. Natl. Adad. Sci. USA* (1999) **96**, 290-295.

The presence of the peptide motif of sequence (I) further defines the members of the GRAS family from which the mutants of the present invention can be obtained. This motif (GYRVEEE or GYNVEE) is present only in a sub-group of GRAS proteins that are involved in regulation of plant size. The sequence alignment shown in the **enclosed** Figure 1 of the present application shows that this motif is present in BZH, GAI, RGA, and RGAL proteins, each of which are involved in the regulation of plant height. In contrast, this motif is not found in SCR, which is involved in radial patterning of the roots, or LS which is involved in shoot branching. Applicants also **submit herewith** Peng et al, *Nature* (1999) **400**, 256-261, which discloses GRAS proteins involved in regulation of plant size in wheat and maize and which containing the GYRVEE sequence (maize d8) or a closely related sequence (GYKVEE in the case of wheat Rht-1).

The mutants of the present invention are defined by a mutation in the aforementioned conserved sequence, namely, by a substitution of the C-terminal glutamic acid by a basic amino acid. Due to the high degree of conservation of this glutamic acid residue in GRAS

proteins involved in the regulation of plant size, it is highly probably that the same mutation in any of these proteins will result in a reduction of the plant size.

In view of the foregoing, Applicants submit that the specification provides an adequate description, when coupled with the state of the art at the time of the present invention, to allow the skilled artisan to recognize what has been invented. As such, what is claimed is adequately described in the specification within the meaning of 35 U.S.C. § 112, first paragraph.

Withdrawal of this ground of rejection is requested.

The rejections of Claims 1-11 under 35 U.S.C. §112, first paragraph (enablement), are obviated in part by amendment and traversed in part.

The Office has taken the position that the claimed invention is not supported by an enabling disclosure (page 7, first paragraph of the Office Action mailed July 29, 2005). Applicants respectfully disagree.

Further, on page 8, fourth paragraph, of the Office Action mailed July 29, 2005, the Examiner makes a proclamation that "Applicants have not reduced to practice their invention." Applicants submit that the Examiner's proclamation is without any merit. In fact, the mere "filing of a patent application serves as conception and constructive reduction to practice of the subject matter described in the application" (MPEP § 2138.05). Moreover, MPEP §2138.05 states: "the inventor need not provide evidence of either conception or actual reduction to practice when relying on the content of the patent application."

MPEP §2164.04 states:

> A specification disclosure which contains a teaching of the manner and process of making and using an invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented must be taken as being in compliance with the enablement

> requirement of 35 U.S.C. 112, first paragraph, unless there is a reason to doubt the objective truth of the statements contained therein which must be relied on for enabling support.

Throughout the specification, Applicants provide a detailed explanation of how the skilled artisan may clone, express, and characterize the polynucleotides that fall within the scope of the present invention. For the reasons stated above, the identification of the full scope of parent sequences containing the motif of sequence (I) would be readily apparent to the skilled artisan. Further, the information provided in the present specification with respect to the nature of the gene, the nature and position of the mutations, and the effect of this mutation (reduction in size depending on the level of expression in the plant of the mutant sequence), is sufficient to place the skilled artisan in possession of the full scope of the present invention to reliably reproduce the same. Moreover, Applicants submit that procedures for expressing a gene in a plant were well known to the skilled artisan as of the date of the present invention. As such, the skilled artisan could reliably use the procedures available in the art for expressing the mutant gent of the present invention without undue experimentation.

MPEP § 2164.01 states:

> The test of enablement is whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent coupled with information known in the art without undue experimentation.

Applicants submit that determining what sequences fall within or without the scope of the present claims, as well as producing plants expressing the same, would be readily apparent to the skilled artisan with the present application in hand. This is especially true when the state of the art as represented by Pysh et al, Schumacher et al, and Peng et al is considered.

Based on the foregoing, Applicants submit that the present claims are fully enabled by the specification and the common knowledge available in the art and as such withdrawal of this ground of rejection is requested.

In view of the foregoing, Applicants request withdrawal of these grounds of rejection.

The rejection of Claims 1-11 under 35 U.S.C. §102(b) over Foisset et al is respectfully traversed.

The Examiner alleges that Foisset et al disclose a dwarf *Brassica napus* plant comprising a mutant *breizh* (*bzh*) gene. The Examiner's bases this allegation on the statement on page 3, lines 30-33 in the present specification. The Examiner further alleges that Applicants' claims read on an endogenous gene and not to a gene that that has been isolated.

The allegation that Applicants' claims read on an endogenous gene is obviated by the amendment to the claims to add the term "isolated." As such, this criticism is no longer tenable. It is also believed that this rejection obviates this rejection in its entirety as each of Claims 2-8 and 11-16 depend from Claim 1 and Foisset et al fail to disclose or suggest any isolated polynucleotides much less that set forth in Claim 1.

Applicants submit that the claimed invention is also novel in view of Foisset et al as this reference fails to enable an isolated mutant polynucleotide meeting the limitations of Claim 1 or plants expressing the same. The Examiner is reminded:

> "In determining that quantum of prior art disclosure which is necessary to declare an applicant's invention 'not novel' or 'anticipated' within section 102, the stated test is whether a reference contains an 'enabling disclosure'... ." *In re Hoeksema,* 399 F.2d 269, 158 USPQ 596 (CCPA 1968). The disclosure in an assertedly anticipating reference must provide an enabling disclosure of the desired subject matter; mere naming or description of the subject matter is insufficient, if it cannot be produced without undue experimentation. *Elan Pharm., Inc. v. Mayo Found. For Med. Educ.*

& Research<, 346 F.3d 1051, 1054, 68 USPQ2d 1373, 1376 (Fed. Cir. 2003) (MPEP §2121.01)

The Examiner alleges that Applicants admit that the phenotype observed in Foisset et al is the result of a mutant *bzh* gene. However, Applicants submit that the Foisset et al fails to provide any evidence to suggest that the resultant dwarf phenotype is the result of a mutant *bzh* gene, it is only the present invention that has isolated, characterized, and determined the link between dwarfism and the specifically claimed mutant *bzh* gene.

Foisset et al disclose that they produced a rapeseed dwarf mutant from chemical (EMS) mutagenesis. However, Applicants submit that such a disclosure is not sufficient to reproduce the claimed invention. If the skilled artisan were to follow the disclosure of Foisset et al and perform chemical mutagenesis in accordance therewith, a number of dwarf mutants would likely result. There are several genes that can be involved in dwarfism. For example, Applicants **submit herewith** Annex I, which provides abstract of some publications showing that a large variety of genes belonging to the gibberellin pathway or the brassinosteroids pathway may be involved in dwarfism.

Even with the *bzh* gene there are several mutations in this gene that may induce dwarfism (e.g., mutations in the N-terminal portion of the protein as disclosed in Peng et al for *Arabidopsis gai* gene). Foisset et al disclose that the *bzh* mutation has been obtained by EMS mutagenesis of seeds. EMS mutagenesis primarily induces G→A substitutions. Therefore, the only suggestion that the skilled artisan would take from Foisset et al is that the *bzh* mutation is likely a G→A substitution. However, the size of the rapeseed genome is about 1200 x 10^6 bp. If one considers a G/C content of approximately 50%, there would be about 600 x 10^6 possible G→A substitutions genome-wide.

The only "guide" that Foisset et al provide to determine which of the 600 x 10^6 possible G→A substitutions is responsible for the *bzh* mutation is that the *bzh* mutation results in dwarfism. Foisset et al fail to provide any guidance to the skilled artisan to a homolog of the *gai* gene of *Arabidopsis*. Further, Foisset et al do not disclose or suggest that the *bzh* mutation has a characteristic of "semi-dominance" and insensitivity to gibberellins, which are similar to those of the *gai* mutations. Only the present inventors have discovered these characteristics.

As stated above and shown in Annex I a large variety of genes, belonging to (for example) the gibberellin pathway or the brassinosteroids pathway, may be involved in dwarfism. In view of the disclosure of Foisset et al, the skilled artisan would conclude the G→A substitute responsible for the *bzh* mutation is found somewhere in one of the known or unknown genes possibly involved in dwarfism. However, there is no disclosure or suggestion in Foisset et al to direct the skilled artisan to the specific gene or the specifically claimed polynucleotide.

Accordingly, Applicants submit that in view of the disclosure of Foisset et al, the skilled artisan would not have been able to differentiate the dwarf mutant of the present invention from other dwarf mutants obtained via chemical mutagenesis, much less identify the specifically claimed gene. As such, Applicants request withdrawal of this ground of rejection.

The rejection of Claims 1 and 5-7 under 35 U.S.C. §102(b) over Peng et al is obviated by amendment.

Applicants have amended Claim 1 to include the limitations of Claims 2 and 3, which the Examiner recognizes as being free from the disclosure of Peng et al. As such, Peng et al no longer affects the patentability of the present invention.

In view of the present amendment, Applicants request withdrawal of this ground of rejection.

The rejection of Claims 1-11 under 35 U.S.C. §101 is believed to be obviated by the amendments herein. Applicants have amended the claims to ensure that the claimed invention is distinguished from products of nature (i.e., require the 'hand of man'). As such, Applicants request that the Examiner withdraw this ground of rejection.

The objection to Claim 7 is obviated by amendment. Applicants thank the Examiner for bringing these issues to their attention. In accordance with the Examiner's suggestions, Claim 7 has been amended. Withdrawal of this ground of objection is requested.

The objection to the specification, in particular the objection to the brief description of the drawings, is obviated by amendment. Applicants have amended the specification to insert an appropriate description of Figure 1. Withdrawal of this ground of objection is requested.

Finally, the Examiner has objected to the Declaration on two grounds. First, the original Declaration allegedly contains the wrong filing date of the present application and the original Declaration contained non-initialed/non-dated alterations.

In regard to the first point, the Examiner is incorrect as the date on the original Declaration is properly listed as the date upon which the application was actually filed in the

U.S., whereas the date indicated by the Examiner is that of compliance with the requirements of 371. Indeed the original Declaration was in compliance with the requirements of 37 CFR §1.63. Therefore, no change is believed to be necessary.

In regard to the second point, the Examiner notes that Mr. Pierre Barret modified his address, but did not initial and date of the modification. However, Applicants note that Mr. Barret *did* sign and date in the same box as the modification, thus qualifying as an initialed and dated alteration. Therefore, a substitute Declaration is not believed to be necessary.

Despite the foregoing, Applicants are currently attempting to obtain a newly executed substitute Declaration. However, the substitute Declaration is not yet available and will be filed once it is properly executed by the inventors.

Applicants submit that the present application is in condition for allowance. Early notification to this effect is respectfully requested.

Respectfully submitted,

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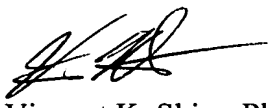
Norman F. Oblon



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SPRAY, et al.: *The dwarf-1 (dt) Mutant of Zea mays blocks three steps in the gibberellin-biosynthetic pathway*; Proc Natl Acad Sci U S A, 93, 10515-10518, 1996
ABSTRACT: In plants, gibberellin (GA)-responding mutants have been used as tools to identify the genes that control specific steps in the GA-biosynthetic pathway. They have also been used to determine which native GAs are active per se, i.e., further metabolism is not necessary for bioactivity. We present metabolic evidence that the D1 gene of maize (Zea mays L.) controls the three biosynthetic steps: GA20 to GA1, Ga20 to GA5, and GA5 to GA3. We also present evidence that three gibberellins, GA1, GA5, and GA3, have per se activity in stimulating shoot elongation in maize. The metabolic evidence comes from the injection of [17-13C,3H]GA20 and [17-13C,3H]GA5 into seedlings of d1 and controls (normal and d5), followed by isolation and identification of the 13C-labeled metabolites by full-scan GC-MS and Kovats retention index. For the controls, GA20 was metabolized to GA1,GA3, and GA5; GA5 was metabolized to GA3. For the d1 mutant, GA20 was not metabolized to GA1, GA3, or to GA5, and GA5 was not metabolized to GA3. The bioassay evidence is based on dosage response curves using d1 seedlings for assay. GA1, GA3, and GA5 had similar bioactivities, and they were 10-times more active than GA20.

MARTIN, et al.: *Mendel's dwarfing gene: cDNAs from the Le alleles and function of the expressed proteins*; Proc Natl Acad Sci U S A, 94, 8907-8911, 1997
ABSTRACT: The major gibberellin (GA) controlling stem elongation in pea (Pisum sativum L.) is GA1, which is formed from GA20 by 3beta-hydroxylation. This step, which limits GA1 biosynthesis in pea, is controlled by the Le locus, one of the original Mendelian loci. Mutations in this locus result in dwarfism. We have isolated cDNAs encoding a GA 3beta-hydroxylase from lines of pea carrying the Le, le, le-3, and led alleles. The cDNA sequences from le and le-3 each contain a base substitution resulting in single amino acid changes relative to the sequence from Le. The cDNA sequence from led, a mutant derived from an le line, contains both the le "mutation" and a single-base deletion, which causes a shift in reading frame and presumably a null mutation. cDNAs from each line were expressed in Escherichia coli. The expression product for the clone from Le converted GA9 to GA4, and GA20 to GA1, with Km values of 1.5 microM and 13 microM, respectively. The amino acid substitution in the clone from le increased Km for GA9 100-fold and reduced conversion of GA20 to almost nil. Expression products from le and le-3 possessed similar levels of 3beta-hydroxylase activity, and the expression product from led was inactive. Our results suggest that the 3beta-hydroxylase cDNA is encoded by Le. Le transcript is expressed in roots, shoots, and cotyledons of germinating pea seedlings, in internodes and leaves of established seedlings, and in developing seeds.

SPONSEL, et al.: *Characterization of new gibberellin-responsive semidwarf mutants of arabidopsis*; Plant Physiol, 115, 1009-1020, 1997
ABSTRACT: Chemical mutagenesis of Arabidopsis thaliana (L.) Heynh. yielded four semidwarf mutants, all of which appeared to be gibberellin (GA)-biosynthesis mutants. All four had atypical response profiles to C20-GAs, suggesting that each had impaired 20-oxidation. One mutant, 11.2, was shown to be allelic to ga5 and has been named ga5-2. It had altered metabolism of [14C]GA15 relative to that in wild-type plants and undetectable levels of C19-GAs in young stems, consistent with the known function of GA5 as a stem-expressed GA 20-oxidase. Two mutants (2.1 and 10.3), which had very short inflorescences and siliques, were allelic to each other but not to the known GA-responding mutants, ga1 to ga5. The locus defined by these two mutations is provisionally named GA6 and is purported to encode an inflorescence- and silique-expressed GA 20-oxidase. A double mutant, ga5-2 ga6-

2, had an extreme dwarf phenotype with very short siliques. The fourth mutation, 1.1, gave a phenotype like ga5, but was not allelic to any of the known ga mutations. It has not yet been given a gene symbol pending further studies.

CHOE, et al.: *The DWF4 gene of Arabidopsis encodes a cytochrome P450 that mediates multiple 22alpha-hydroxylation steps in brassinosteroid biosynthesis*; Plant Cell, 10, 231-243, 1998

ABSTRACT: dwarf4 (dwf4) mutants of Arabidopsis display a dwarfed phenotype due to a lack of cell elongation. Dwarfism could be rescued by the application of brassinolide, suggesting that DWF4 plays a role in brassinosteroid (BR) biosynthesis. The DWF4 locus is defined by four mutant alleles. One of these is the result of a T-DNA insertion. Plant DNA flanking the insertion site was cloned and used as a probe to isolate the entire DWF4 gene. Sequence analysis revealed that DWF4 encodes a cytochrome P450 monooxygenase with 43% identity to the putative Arabidopsis steroid hydroxylating enzyme. Sequence analysis of two other mutant alleles revealed deletions or a premature stop codon, confirming that DWF4 had been cloned. This sequence similarity suggests that DWF4 functions in specific hydroxylation steps during BR biosynthesis. In fact, feeding studies utilizing BR intermediates showed that only 22alpha-hydroxylated BRs rescued the dwf4 phenotype, confirming that DWF4 acts as a 22alpha-hydroxylase.

ASHIKARI, et al.: *Rice gibberellin-insensitive dwarf mutant gene Dwarf 1 encodes the alpha-subunit of GTP-binding protein*; Proc Natl Acad Sci U S A, 96, 10284-10289, 1999

ABSTRACT: A rice Dwarf 1 gene was identified by using a map-based cloning strategy. Its recessive mutant allele confers a dwarf phenotype. Linkage analysis revealed that a cDNA encoding the alpha-subunit of GTP-binding protein cosegregated with d1 in 3,185 d1 segregants. Southern hybridization analysis with this cDNA as a probe showed different band patterns in several d1 mutant lines. In at least four independent d1 mutants, no gene transcript was observed by Northern hybridization analysis. Sequencing analysis revealed that an 833-bp deletion had occurred in one of the mutant alleles, which resulted in an inability to express GTP-binding protein. A transgenic d1 mutant with GTP-binding protein gene restored the normal phenotype. We conclude that the rice Dwarf 1 gene encodes GTP-binding protein and that the protein plays an important role in plant growth and development. Because the d1 mutant is classified as gibberellin-insensitive, we suggest that the GTP-binding protein might be associated with gibberellin signal transduction.

CHOE, et al.: *The Arabidopsis dwarf1 mutant is defective in the conversion of 24-methylenecholesterol to campesterol in brassinosteroid biosynthesis*; Plant Physiol, 119, 897-907, 1999

ABSTRACT: Since the isolation and characterization of dwarf1-1 (dwf1-1) from a T-DNA insertion mutant population, phenotypically similar mutants, including deetiolated2 (det2), constitutive photomorphogenesis and dwarfism (cpd), brassinosteroid insensitive1 (bri1), and dwf4, have been reported to be defective in either the biosynthesis or the perception of brassinosteroids. We present further characterization of dwf1-1 and additional dwf1 alleles. Feeding tests with brassinosteroid-biosynthetic intermediates revealed that dwf1 can be rescued by 22alpha-hydroxycampesterol and downstream intermediates in the brassinosteroid pathway. Analysis of the endogenous levels of brassinosteroid intermediates showed that 24-methylenecholesterol in dwf1 accumulates to 12 times the level of the wild type, whereas the level of campesterol is greatly diminished, indicating that the defective step is in C-24 reduction. Furthermore, the deduced amino acid sequence of DWF1 shows significant similarity to a flavin adenine dinucleotide-binding domain conserved in various

oxidoreductases, suggesting an enzymatic role for DWF1. In support of this, 7 of 10 dwf1 mutations directly affected the flavin adenine dinucleotide-binding domain. Our molecular characterization of dwf1 alleles, together with our biochemical data, suggest that the biosynthetic defect in dwf1 results in reduced synthesis of bioactive brassinosteroids, causing dwarfism.

FRIDBORG, et al.: *The Arabidopsis dwarf mutant shi exhibits reduced gibberellin responses conferred by overexpression of a new putative zinc finger protein*; Plant Cell, 11, 1019-1032, 1999

ABSTRACT: shi (for short internodes), a semidominant dwarfing mutation of Arabidopsis caused by a transposon insertion, confers a phenotype typical of mutants defective in the biosynthesis of gibberellin (GA). However, the application of GA does not correct the dwarf phenotype of shi plants, suggesting that shi is defective in the perception of or in the response to GA. In agreement with this observation, the level of active GAs was elevated in shi plants, which is the result expected when feedback control of GA biosynthesis is reduced. Cloning of the SHI gene revealed that in shi, the transposon is inserted into the untranslated leader so that a cauliflower mosaic virus 35S promoter in the transposon reads out toward the SHI open reading frame. This result, together with mRNA analysis, suggests that the phenotype of the shi mutant is a result of overexpression of the SHI open reading frame. The predicted amino acid sequence of SHI has acidic and glutamine-rich stretches and shows sequence similarity over a putative zinc finger region to three presumptive Arabidopsis proteins. This suggests that SHI may act as a negative regulator of GA responses through transcriptional control.

FUJISAWA, et al.: *Suppression of the heterotrimeric G protein causes abnormal morphology, including dwarfism, in rice*; Proc Natl Acad Sci U S A, 96, 7575-7580, 1999

ABSTRACT: Transgenic rice containing an antisense cDNA for the alpha subunit of rice heterotrimeric G protein produced little or no mRNA for the subunit and exhibited abnormal morphology, including dwarf traits and the setting of small seeds. In normal rice, the mRNA for the alpha subunit was abundant in the internodes and florets, the tissues closely related to abnormality in the dwarf transformants. The position of the alpha-subunit gene was mapped on rice chromosome 5 by mapping with the restriction fragment length polymorphism. The position was closely linked to the locus of a rice dwarf mutant, Daikoku dwarf (d-1), which is known to exhibit abnormal phenotypes similar to those of the transformants that suppressed the endogenous mRNA for the alpha subunit by antisense technology. Analysis of the cDNAs for the alpha subunits of five alleles of Daikoku dwarf (d-1), ID-1, DK22, DKT-1, DKT-2, and CM1361-1, showed that these dwarf mutants had mutated in the coding region of the alpha-subunit gene. These results show that the G protein functions in the formation of normal internodes and seeds in rice.

CHOE, et al.: *Lesions in the sterol delta reductase gene of Arabidopsis cause dwarfism due to a block in brassinosteroid biosynthesis*; Plant J, 21, 431-443, 2000

ABSTRACT: The brassinosteroid (BR) biosynthetic pathway, and the sterol pathway which is prerequisite to the BR pathway, are rapidly being characterized because of the availability of a large number of characteristic dwarf mutants in Arabidopsis. Here we show that the Arabidopsis dwarf5 mutants are disrupted in a sterol Delta7 reduction step. dwf5 plants display the characteristic dwarf phenotype typical of other BR mutants. This phenotype includes small, round, dark-green leaves, and short stems, pedicels, and petioles. Metabolite tracing with 13C-labeled precursors in dwf5 verified a deficiency in a sterol Delta7 reductase activity. All six independent alleles contain loss-of-function mutations in the sterol Delta7 reductase gene. These include a putative mRNA instability mutation in dwf5-1, 3' and 5'

splice-site mutations in dwf5-2 and dwf5-6, respectively, premature stop codons in dwf5-3 (R400Z) and dwf5-5 (R409Z), and a mis-sense mutation in dwf5-4 (D257N). The dwf5 plant could be restored to wild type by ectopic overexpression of the wild-type copy of the gene. Both the Arabidopsis dwf5 phenotype and the human Smith-Lemli-Opitz syndrome are caused by loss-of-function mutations in a sterol Delta7 reductase gene, indicating that it is required for the proper growth and development of these two organisms.

FUJISAWA, et al.: *Structure and function of heterotrimeric G proteins in plants*; Plant Cell Physiol, 42, 789-794, 2001
ABSTRACT: Heterotrimeric G proteins are mediators that transmit the external signals via receptor molecules to effector molecules. The G proteins consist of three different subunits: alpha, beta, and gamma subunits. The cDNAs or genes for all the alpha, beta, and gamma subunits have been isolated from many plant species, which has contributed to great progress in the study of the structure and function of the G proteins in plants. In addition, rice plants lacking the alpha subunit were generated by the antisense method and a rice mutant, Daikoku d1, was found to have mutation in the alpha-subunit gene. Both plants show abnormal morphology such as dwarfism, dark green leaf, and small round seed. The findings revealed that the G proteins are functional molecules regulating some body plans in plants. There is evidence that the plant G proteins participate at least in signaling of gibberellin at low concentrations. In this review, we summarize the currently known information on the structure of plant heterotrimeric G proteins and discuss the possible functions of the G proteins in plants.

BOSS and THOMAS: *Association of dwarfism and floral induction with a grape 'green revolution' mutation*; Nature, 416, 847-850, 2002
ABSTRACT: The transition from vegetative to reproductive growth is an essential process in the life cycle of plants. Plant floral induction pathways respond to both environmental and endogenous cues and much has been learnt about these genetic pathways by studying mutants of Arabidopsis. Gibberellins (GAs) are plant growth regulators important in many aspects of plant growth and in Arabidopsis they promote flowering. Here we provide genetic evidence that GAs inhibit flowering in grapevine. A grapevine dwarf mutant derived from the L1 cell layer of the champagne cultivar Pinot Meunier produces inflorescences along the length of the shoot where tendrils are normally formed. The mutated gene associated with the phenotype is a homologue of the wheat 'green revolution' gene Reduced height-1 (ref. 6) and the Arabidopsis gene GA insensitive (GAI). The conversion of tendrils to inflorescences in the mutant demonstrates that the grapevine tendril is a modified inflorescence inhibited from completing floral development by GAs.

CHOE, et al.: *Arabidopsis brassinosteroid-insensitive dwarf12 mutants are semidominant and defective in a glycogen synthase kinase 3beta-like kinase*; Plant Physiol, 130, 1506-1515, 2002
ABSTRACT: Mutants defective in the biosynthesis or signaling of brassinosteroids (BRs), plant steroid hormones, display dwarfism. Loss-of-function mutants for the gene encoding the plasma membrane-located BR receptor BRI1 are resistant to exogenous application of BRs, and characterization of this protein has contributed significantly to the understanding of BR signaling. We have isolated two new BR-insensitive mutants (dwarf12-1D and dwf12-2D) after screening Arabidopsis ethyl methanesulfonate mutant populations. dwf12 mutants displayed the characteristic morphology of previously reported BR dwarfs including short stature, short round leaves, infertility, and abnormal de-etiolation. In addition, dwf12 mutants exhibited several unique phenotypes, including severe downward curling of the leaves.

Genetic analysis indicates that the two mutations are semidominant in that heterozygous plants show a semidwarf phenotype whose height is intermediate between wild-type and homozygous mutant plants. Unlike BR biosynthetic mutants, dwf12 plants were not rescued by high doses of exogenously applied BRs. Like bri1 mutants, dwf12 plants accumulated castasterone and brassinolide, 43- and 15-fold higher, respectively, providing further evidence that DWF12 is a component of the BR signaling pathway that includes BRI1. Map-based cloning of the DWF12 gene revealed that DWF12 belongs to a member of the glycogen synthase kinase 3beta family. Unlike human glycogen synthase kinase 3beta, DWF12 lacks the conserved serine-9 residue in the auto-inhibitory N terminus. In addition, dwf12-1D and dwf12-2D encode changes in consecutive glutamate residues in a highly conserved TREE domain. Together with previous reports that both bin2 and ucu1 mutants contain mutations in this TREE domain, this provides evidence that the TREE domain is of critical importance for proper function of DWF12/BIN2/UCU1 in BR signal transduction pathways.

MONNA, et al.: *Positional cloning of rice semidwarfing gene, sd-1: rice "green revolution gene" encodes a mutant enzyme involved in gibberellin synthesis*; DNA Res, 9, 11-17, 2002
ABSTRACT: A rice semidwarfing gene, sd-1, known as the "green revolution gene," was isolated by positional cloning and revealed to encode gibberellin 20-oxidase, the key enzyme in the gibberellin biosynthesis pathway. Analysis of 3477 segregants using several PCR-based marker technologies, including cleaved amplified polymorphic sequence, derived-CAPS, and single nucleotide polymorphisms revealed 1 ORF in a 6-kb candidate interval. Normal-type rice cultivars have an identical sequence in this region, consisting of 3 exons (558, 318, and 291 bp) and 2 introns (105 and 1471 bp). Dee-Geo-Woo-Gen-type sd-1 mutants have a 383-bp deletion from the genome (278-bp deletion from the expressed sequence), from the middle of exon 1 to upstream of exon 2, including a 105-bp intron, resulting in a frame-shift that produces a termination codon after the deletion site. The radiation-induced sd-1 mutant Calrose 76 has a 1-bp substitution in exon 2, causing an amino acid substitution (Leu [CTC] to Phe [TTC]). Expression analysis suggests the existence of at least one more locus of gibberellin 20-oxidase which may prevent severe dwarfism from developing in sd-1 mutants.

BUSOV, et al.: *Activation tagging of a dominant gibberellin catabolism gene (GA 2-oxidase) from poplar that regulates tree stature*; Plant Physiol, 132, 1283-1291, 2003
ABSTRACT: We identified a dwarf transgenic hybrid poplar (Populus tremula x Populus alba) after screening of 627 independent activation-tagged transgenic lines in tissue culture, greenhouse, and field environments. The cause of the phenotype was a hyperactivated gene encoding GA 2-oxidase (GA2ox), the major gibberellin (GA) catabolic enzyme in plants. The mutation resulted from insertion of a strong transcriptional enhancer near the transcription start site. Overexpression of the poplar GA2ox gene (PtaGA2ox1) caused hyperaccumulation of mRNA transcripts, quantitative shifts in the spectrum of GAs, and similarity in phenotype to transgenic poplars that overexpress a bean (Phaseolus coccineus) GA2ox gene. The poplar PtaGA2ox1 sequence was most closely related to PsGA2ox2 from pea (Pisum sativum) and two poorly known GA2oxs from Arabidopsis (AtGA2ox4 and AtGA2ox5). The dwarf phenotype was reversible through gibberellic acid application to the shoot apex. Transgenic approaches to producing semidwarf trees for use in arboriculture, horticulture, and forestry could have significant economic and environmental benefits, including altered fiber and fruit production, greater ease of management, and reduced risk of spread in wild populations.

SALAMINI: *Plant Biology. Hormones and the green revolution*; Science, 302, 71-72, 2003

ABSTRACT: The success of the green revolution largely resulted from the creation of dwarf cultivars of wheat and rice, which had much higher yields than conventional crops. Characterization of these dwarf cultivars showed that the mutant genes were involved in either the synthesis or signaling of gibberellin, a plant growth hormone. In his Perspective, Salamini highlights new work (Multani et al.) that identifies the cause of dwarfism in agronomically important varieties of maize and sorghum. In these cases, dwarfism is caused by defective transport of another growth hormone called auxin.

SASAKI, et al.: *Accumulation of phosphorylated repressor for gibberellin signaling in an F-box mutant*; Science, 299, 1896-1898, 2003
ABSTRACT: Gibberellin (GA) regulates growth and development in plants. We isolated and characterized a rice GA-insensitive dwarf mutant, gid2. The GID2 gene encodes a putative F-box protein, which interacted with the rice Skp1 homolog in a yeast two-hybrid assay. In gid2, a repressor for GA signaling, SLR1, was highly accumulated in a phosphorylated form and GA increased its concentration, whereas SLR1 was rapidly degraded by GA through ubiquitination in the wild type. We conclude that GID2 is a positive regulator of GA signaling and that regulated degradation of SLR1 is initiated through GA-dependent phosphorylation and finalized by an SCF(GID2)-proteasome pathway.

SCHOMBURG, et al.: *Overexpression of a novel class of gibberellin 2-oxidases decreases gibberellin levels and creates dwarf plants*; Plant Cell, 15, 151-163, 2003
ABSTRACT: Degradation of active C(19)-gibberellins (GAs) by dioxygenases through 2beta-hydroxylation yields inactive GA products. We identified two genes in Arabidopsis (AtGA2ox7 and AtGA2ox8), using an activation-tagging mutant screen, that encode 2beta-hydroxylases. GA levels in both activation-tagged lines were reduced significantly, and the lines displayed dwarf phenotypes typical of mutants with a GA deficiency. Increased expression of either AtGA2ox7 or AtGA2ox8 also caused a dwarf phenotype in tobacco, indicating that the substrates for these enzymes are conserved. AtGA2ox7 and AtGA2ox8 are more similar to each other than to other proteins encoded in the Arabidopsis genome, indicating that they may constitute a separate class of GA-modifying enzymes. Indeed, enzymatic assays demonstrated that AtGA2ox7 and AtGA2ox8 both perform the same GA modification: 2beta-hydroxylation of C(20)-GAs but not of C(19)-GAs. Lines containing increased expression of AtGA2ox8 exhibited a GA dose-response curve for stem elongation similar to that of the biosynthetic mutant ga1-11. Double loss-of-function Atga2ox7 Atga2ox8 mutants had twofold to fourfold higher levels of active GAs and displayed phenotypes associated with excess GAs, such as early bolting in short days, resistance to the GA biosynthesis inhibitor ancymidol, and decreased mRNA levels of AtGA20ox1, a gene in the GA biosynthetic pathway.

SUZUKI, et al.: *A dwarf mutant strain of Pharbitis nil, Uzukobito (kobito), has defective brassinosteroid biosynthesis*; Plant J, 36, 401-410, 2003
ABSTRACT: Japanese morning glory (Pharbitis nil) is a model plant characterized by a large stock of spontaneous mutants. The recessive mutant Uzukobito shows strong dwarfism with dark-green rugose leaves. The phenotype was rescued by the application of brassinolide, a bioactive brassinosteroid (BR), indicating that Uzukobito was a BR-deficient mutant. A detailed analysis of the endogenous BR levels in Uzukobito and its parental wild-type plant showed that Uzukobito had a lower level of BRs downstream of (24R)-24-methyl-5alpha-cholestan-3-one and (22S, 24R)-22-hydroxy-24-methyl-5alpha-cholestan-3-one than those in wild-type plants, while their immediate precursors (24R)-24-methylcholest-4-en-3-one and (22S, 24R)-22-hydroxy-24-methylcholest-4-en-3-one accumulated relatively more in

Uzukobito. These results indicate that Uzukobito had a defect in the conversion of (24R)-24-methylcholest-4-en-3-one and (22S, 24R)-22-hydroxy-24-methylcholest-4-en-3-one to their 5alpha-reduced forms, which is catalyzed by de-etiolated2 (DET2) in Arabidopsis. The P. nil ortholog of the DET2 gene (PnDET2) was cloned and shown to have the greatest similarity to DET2 among all the putative genes in Arabidopsis. Uzukobito had one amino acid substitution from Glu62 to Val62 in the deduced amino acid sequence of PnDET2. Recombinant PnDET2 expressed in COS-7 cells was found to be a functional steroid 5alpha-reductase (S5alphaR) converting (24R)-24-methylcholest-4-en-3-one to (24R)-24-methyl-5alpha-cholestan-3-one, while PnDET2 with the mutation did not show any catalytic activity. This shows that a plant S5alphaR can convert an intrinsic substrate. All these results clearly demonstrate that the Uzukobito phenotype resulted from a mutation on PnDET2, and a morphological mutant has been characterized at the molecular level among a large stock of P. nil mutants.

BROCARD-GIFFORD, et al.: *The Arabidopsis thaliana ABSCISIC ACID-INSENSITIVE8 encodes a novel protein mediating abscisic acid and sugar responses essential for growth*; Plant Cell, 16, 406-421, 2004

ABSTRACT: Abscisic acid (ABA) regulates many aspects of plant growth and development, yet many ABA response mutants present only subtle phenotypic defects, especially in the absence of stress. By contrast, the ABA-insensitive8 (abi8) mutant, isolated on the basis of ABA-resistant germination, also displays severely stunted growth, defective stomatal regulation, altered ABA-responsive gene expression, delayed flowering, and male sterility. The stunted growth of the mutant is not rescued by gibberellin, brassinosteroid, or indoleacetic acid application and is not attributable to excessive ethylene response, but supplementing the medium with Glc improves viability and root growth. In addition to exhibiting Glc-dependent growth, reflecting decreased expression of sugar-mobilizing enzymes, abi8 mutants are resistant to Glc levels that induce developmental arrest of wild-type seedlings. Studies of genetic interactions demonstrate that ABA hypersensitivity conferred by the ABA-hypersensitive1 mutation or overexpression of ABI3 or ABI5 does not suppress the dwarfing and Glc dependence caused by abi8 but partially suppresses ABA-resistant germination. By contrast, the ABA-resistant germination of abi8 is epistatic to the hypersensitivity caused by ethylene-insensitive2 (ein2) and ein3 mutations, yet ABI8 appears to act in a distinct Glc response pathway from these EIN loci. ABI8 encodes a protein with no domains of known function but belongs to a small plant-specific protein family. Database searches indicate that it is allelic to two dwarf mutants, elongation defective1 and kobito1, previously shown to disrupt cell elongation, cellulose synthesis, vascular differentiation, and root meristem maintenance. The cell wall defects appear to be a secondary effect of the mutations because Glc treatment restores root growth and vascular differentiation but not cell elongation. Although the ABI8 transcript accumulates in all tested plant organs in both wild-type and ABA response mutants, an ABI8-beta-glucuronidase fusion protein is localized primarily to the elongation zone of roots, suggesting substantial post-transcriptional regulation of ABI8 accumulation. This localization pattern is sufficient to complement the mutation, indicating that ABI8 acts either at very low concentrations or over long distances within the plant body.

ITOH, et al.: *A rice semi-dwarf gene, Tan-Ginbozu (D35), encodes the gibberellin biosynthesis enzyme, ent-kaurene oxidase*; Plant Mol Biol, 54, 533-547, 2004

ABSTRACT: A rice (Oryza sativa L.) semi-dwarf cultivar, Tan-Ginbozu (d35Tan-Ginbozu), contributed to the increase in crop productivity in Japan in the 1950s. Previous studies suggested that the semi-dwarf stature of d35Tan-Ginbozu is caused by a defective early step

of gibberellin biosynthesis, which is catalyzed by ent-kaurene oxidase (KO). To study the molecular characteristics of d35Tan-Ginbozu, we isolated 5 KO-like (KOL) genes from the rice genome, which encoded proteins highly homologous to Arabidopsis and pumpkin KOs. The genes (OsKOL1 to 5) were arranged as tandem repeats in the same direction within a 120 kb sequence. Expression analysis revealed that OsKOL2 and OsKOL4 were actively transcribed in various organs, while OsKOL1 and OsKOL5 were expressed only at low levels; OsKOL3 may be a pseudogene. Sequence analysis and complementation experiments demonstrated that OsKOL2 corresponds to D35. Homozygote with null alleles of D35 showed a severe dwarf phenotype; therefore, d35Tan-Ginbozu is a weak allele of D35. Introduction of OsKOL4 into d35Tan-Ginbozu did not rescue its dwarf phenotype, indicating that OsKOL4 is not involved in GA biosynthesis. OsKOL4 and OsKOL5 are likely to take part in phytoalexin biosynthesis, because their expression was promoted by UV irradiation and/or elicitor treatment. Comparing d35Tan-Ginbozu with other high yielding cultivars, we discuss strategies to produce culm architectures suitable for high crop yield by decreasing GA levels.

MAGOME, et al.: *dwarf and delayed-flowering 1, a novel Arabidopsis mutant deficient in gibberellin biosynthesis because of overexpression of a putative AP2 transcription factor*; Plant J, 37, 720-729, 2004

ABSTRACT: A novel gibberellin (GA)-deficient mutant designated dwarf and delayed-flowering 1 (ddf1) was isolated from a library of activation-tagged Arabidopsis. This mutant showed dwarfism and late-flowering, but the phenotype was rescued by exogenous GA3 like known mutants defective in GA biosynthesis. The contents of bioactive GA4 and GA1 were in fact decreased in ddf1 at least partially through the repression of biosynthetic steps catalyzed by GA 20-oxidase (GA20ox). Genetic and molecular analyses revealed that the ddf1 phenotypes are caused by increased or ectopic expression of a putative AP2 transcription factor. Overexpression of DDF2, encoding another putative AP2 transcription factor closely related to DDF1, also conferred the ddf1-like phenotype. Among genes encoding (putative) AP2 transcription factors in Arabidopsis, DDFs are phylogenetically close to dehydration-responsive element binding protein (DREB1)/C-repeat binding factor (CBF) genes, which are known to be involved in stress responses. The ddf1 mutation upregulates a stress-related gene RD29A. DDF1 mRNA is strongly induced by high-salinity stress within 1 h. Moreover, transgenic plants overexpressing DDF1 showed increased tolerance to high-salinity stress. These results suggest that DDF1 is involved in the regulation of GA biosynthesis and stress tolerance. The possible relation between the contents of endogenous GAs and acquisition of stress protection is discussed.

OIKAWA, et al.: *A role of OsGA20ox1 , encoding an isoform of gibberellin 20-oxidase, for regulation of plant stature in rice*; Plant Mol Biol, 55, 687-700, 2004

ABSTRACT: Gibberellin (GA) 20-oxidase (GA20ox) is a key enzyme that normally catalyzes the penultimate steps in GA biosynthesis. One of the GA20ox genes in rice (Oryza sativa L.), OsGA20ox2 (SD1), is well known as the "Green Revolution gene", and loss-of function mutation in this locus causes semi-dwarfism. Another GA20ox gene, OsGA20ox1, has also been identified, but its contribution to plant stature has remained unclear because no suitable mutants have been available. We isolated a mutant, B142, tagged with a T-DNA containing three CaMV 35S promoters, which showed a tall, GA-overproduction phenotype. The final stature of the B142 mutant reflects internode overgrowth and is approximately twice that of its wild-type parent. This mutant responds to application of both GA3 and a GA biosynthesis inhibitor, indicating that it is a novel tall mutant of rice distinct from GA signaling mutants such as slr1 . The integrated T-DNAs, which contain three CaMV 35S

promoters, are located upstream of the OsGA20ox1 open reading frame (ORF) in the B142 mutant genome. Analysis of mRNA and the endogenous GAs reveal that biologically active GA level is increased by up-regulation of the OsGA20ox1 gene in B142. Introduction of OsGA20ox1 cDNA driven by 35S promoter into the wild type phenocopies the morphological characteristics of B142. These results indicate that the elongated phenotype of the B142 mutant is caused by up-regulation of the OsGA20ox1 gene. Moreover, the final stature of rice was reduced by specific suppression of the OsGA20ox1 gene expression. This result indicates that not only OsGA20ox2 but also OsGA20ox1 affects plant stature.

ALCAZAR, et al.: *Overexpression of ADC2 in Arabidopsis induces dwarfism and late-flowering through GA deficiency*; Plant J, 43, 425-436, 2005
ABSTRACT: We have obtained Arabidopsis thaliana transgenic plants constitutively overexpressing ADC2, one of the two genes encoding arginine decarboxylase (ADC) in Arabidopsis. These plants contained very high levels of putrescine (Put) but no changes were observed in spermidine and spermine contents. The results obtained from quantification of free and conjugated polyamines suggest that conjugation may be a limiting step for control of Put homeostasis within a non-toxic range for plant survival. Transgenic plants with increased levels of ADC2 transcript and elevated Put content showed dwarfism and late-flowering, and the phenotype was rescued by gibberellin A3 (GA3) application. The contents of bioactive GA4 and GA1, and of GA9 (a precursor of GA4), as well as the levels of AtGA20ox1, AtGA3ox1 and AtGA3ox3 transcripts (quantified by real-time PCR) were lower in the ADC2 overexpressor plants than in the wild type. No change in the expression of genes encoding earlier enzymes in the GA biosynthesis pathway was detected by microarray analysis. These results suggest that Put accumulation affects GA metabolism through the repression of biosynthetic steps catalyzed by GA 20-oxidase and GA 3-oxidase.

MARGIS-PINHEIRO, et al.: *Isolation and characterization of a Ds-tagged rice (Oryza sativa L.) GA-responsive dwarf mutant defective in an early step of the gibberellin biosynthesis pathway*; Plant Cell Rep, 23, 819-833, 2005
ABSTRACT: We have isolated a severe dwarf transposon (Ds) insertion mutant in rice (Oryza sativa L.), which could be differentiated early in the seedling stage by reduced shoot growth and dark green leaves, and later by severe dwarfism and failure to initiate flowering. These mutants, however, showed normal seed germination and root growth. One of the sequences flanking Ds, rescued from the mutant, was of a chromosome 4-located putative ent-kaurene synthase (KS) gene, encoding the enzyme catalyzing the second step of the gibberellin (GA) biosynthesis pathway. Dwarf mutants were always homozygous for this Ds insertion and no normal plants homozygous for this mutation were recovered in the segregating progeny, indicating that the Ds insertion mutation is recessive. As mutations in three recently reported rice GA-responsive dwarf mutant alleles and the dwarf mutation identified in this study mapped to the same locus, we designate the corresponding gene OsKS1. The osks1 mutant seedlings were responsive to exogenous gibberellin (GA3). OsKS1 transcripts of about 2.3 kb were detected in leaves and stem of wild-type plants, but not in germinating seeds or roots, suggesting that OsKS1 is not involved in germination or root growth. There are at least five OsKS1-like genes in the rice genome, four of which are also represented in rice expressed sequence tag (EST) databases. All OsKS1-like genes are transcribed with different expression patterns. ESTs corresponding to all six OsKS genes are represented in other cereal databases including barley, wheat and maize, suggesting that they are biologically active.

MGLINETS and OSIPOVA: *[dw2, a new mutation of beet Beta vulgaris L.]*; Genetika, 41, 657-660, 2005

ABSTRACT: Twelve dwarf plants were found in the second hybrid generation of beet. The average height of mutant plants was 21.8 cm, their leaf blades and flowers were significantly smaller than normal, and the plants exhibited male and female sterility. This dwarfism was shown to be caused by a mutation differing from that previously described in beet, which is named dwarf2 (dw2). The experimental evidence suggests that this mutation appeared in one of the first-generation plants. Based on plant phenotype in the first hybrid generation and the number of mutant plants in the second one, this mutation is suggested to be under recessive monogenic control of the dw2 gene. The genotypic class segregation in the second hybrid generation indicates that the dw2 gene is inherited independently of genes m, at, and ap that control choricarpousness, gene male sterility, and pollen grain aggregation into tetrads.

MUSSIG: *Brassinosteroid-promoted growth*; Plant Biol (Stuttg), 7, 110-117, 2005

ABSTRACT: Brassinosteroids (BRs) are highly potent growth-promoting sterol derivatives. BR-deficient or BR-insensitive mutants display dwarfism. Whole plants and excised tissues have been used to analyse the mechanisms involved in BR-promoted growth. BR stimulates cell elongation and cell division, and BR has specific effects on differentiation. Underlying physiological pathways include modification of cell wall properties, effects on carbohydrate assimilation and allocation, and control of aquaporin activities. BR apparently coordinates and integrates diverse processes required for growth, partly via interactions with other phytohormones setting the frame for BR responses. Ultimately, BR-promoted growth is mediated through genomic pathways. Positive regulators of the BR response (such as BZR1 and BES1) and putative downstream components (such as EXO) are involved in the regulation of BR-responsive genes and growth promotion. BR-responsive genes have been identified in several plant species. However, causal links between physiological effects and changes of transcript patterns, for the most part, are still unresolved. This review focuses on physiology and molecular mechanisms underlying BR-promoted growth in the different plant organs. Interactions with other phytohormones are discussed.